PHYSIOLOGY

☑ Sheet ○ Slide ○ Handout

Number

1

Subject

Introduction to The Urinary System

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**** This sheet was written according to the recording that belongs to section 3. Please pay attention that <u>the order of ideas in this sheet is a little bit different from that in the recording</u>.**

❖ Before we start,

By the end of the physiology lectures, you should be able to determine the following:

- Whether the kidney is diseased or not.
- What are the tests that must be done to assess kidney function, diagnose kidney diseases, and determine the stage of the renal disease.
  (Kidney Function Tests constitute an essential part in the physiology course of the urinary system).

❖ Topics of this lecture:

*Functions of the kidneys.

*Blood flow to the kidneys.

*Parts of the nephron.

*Processes involved in urine formation (filtration, reabsorption, and secretion).

*How to measure Renal Plasma Flow (RPF) and Renal Blood Flow (RBF).

❖ <u>Functions of the kidneys</u>

There's no doubt that the kidneys are essential for life. They exert several functions, and today, we'll mention some of them.

(1) The kidneys play a key role in **<u>homeostasis of electrolytes</u>**. (We are specifically concerned with K^+, and to a lesser extent, with Ca^{+2}).

Kidney disease → Hyperkalemia (High K^+ in blood)
→ Hyperkalemia can disrupt the normal electrical behavior of the heart and might even stop it (cause cardiac arrest).

Note: a patient with kidney disease, blood potassium above 7 mmol/L, and specific ECG changes must undergo hemodialysis, otherwise, he will die.

<u>Additional piece of information</u>

Normal blood potassium level is 3.6 to 5.2 millimoles per liter (mmol/L).

(2) The kidneys play a key role in **homeostasis of body fluids.**

Kidney disease →Retention of body fluids
→Hypervolemia
→Hypervolemia can induce many problems like pulmonary edema, brain edema, and hypertension.

Kidney disease can cause hypertension. And hypertension can induce or worsen kidney disease. {positive feedback}

(3) The kidneys **secrete erythropoietin (EPO)**, and the stimulus for this process is hypoxia.

Kidney disease → No EPO
→ No RBCs production
→ Anemia, and the patient might die from it.

Recall (from the HLS):

Erythropoietin is a hormone secreted by the kidneys that increases the rate of production of red blood cells in response to falling levels of oxygen in tissues.

(4) The kidneys maintain **acid-base balance** (normal blood pH=7.4).

Kidney disease → acid-base disturbance
The kidneys cannot properly reabsorb bicarbonate and cannot secrete the proper amount of H+ in urine. Retention of H+ will result in acidosis that might kill the patient.

Note: "alkalosis" is also considered a dangerous condition that might cause death.

(5) The kidneys **remove waste products** outside the body.
Urea and creatinine are removed only (exclusively) through the kidney.

Kidney disease →

1. Accumulation of urea in blood. We call this condition "Uremia". Urea is the precursor for ammonia, and ammonia is toxic to brain (brain coma due to ammonia can occur).
2. If the kidney fails, it will not be able to excrete creatinine (which comes from muscles), thus, creatinine level in blood will increase.

Uremia: excess urea in blood (or we can simply say "urine in blood")

The amount of creatinine in blood is **inversely related** to kidneys' function. In other words, high blood creatinine reflects kidney failure.

**Details in the next lecture* but in brief, we can mention the following:*

If normal creatinine is 1mg/dL:

Creatinine level of **2** mg/dl means that 50% of the renal tissue has lost its function (half of the nephrons are lost), and 50% (1/2) remains functional.

Creatinine level of **4** mg/dl would mean that 25% (1/4) of the renal tissue is functional.

(6) The kidneys are the **target of certain hormones**.

For example, Aldosterone, and ADH (Anti-Diuretic Hormone), are secreted in other places (outside the kidney), and travel to the kidneys where they exert their effects.

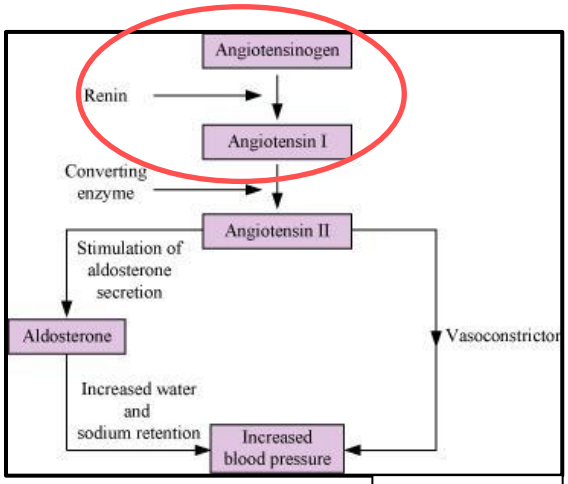
Recall (from the endocrine system):

Aldosterone is a mineralocorticoid hormone, produced by the adrenal cortex in the adrenal gland.

ADH (vasopressin) is stored in, and secreted from, the posterior pituitary.

(7) The kidneys **secrete renin**.

Renin converts angiotensinogen into angiotensin I. In other words, the kidneys participate in regulation of blood pressure (in cases of hypotension, the kidneys participate in returning the blood pressure back to normal).



Extra figure

Conclusion:

Functions of the kidneys are plenty and diverse, and the kidneys are essential for life.

و مَن لم يمُت بالسيف مات بغَيرِهِ ... تعدّدت الأسباب والموت واحدُ

(كان قصد الدكتور: تلف الكلية سَيضرّ الإنسان بطريقةٍ أو بأُخرى)

❖ Blood Flow to the kidneys

- For the kidneys to perform their function, they must receive blood.
- Large amounts of blood enter the kidney (1/4 of the cardiac output).
- The mass of both kidneys together is around 250 grams (أوقيّة), yet they receive 1.25 L of blood per minute (1/4 of the cardiac output). On the other hand, skeletal muscles constitute 40% of the total body weight (approximately 28 Kg in a 70-Kg person), and receive one liter of blood per minute.
  How do we justify the previous statement? Do the kidneys really need such amount of blood for nourishment of the renal tissue (oxygen and nutrients supply)?
  The kidney is a "reconditioning organ". This means that the blood enters the kidneys not only because the kidneys need nourishment, but also because the kidneys will change the composition of the blood (it will "clean" the blood).

Question: How can we prove that the kidney is a reconditioning organ?

Answer: By knowing that the value of the arterio-venous oxygen difference for the kidney is small.

Explanation: arterio-venous oxygen difference (a-vO_2 diff) is the difference in the oxygen content of the blood between the arterial blood and venous blood. It is an indication of how much oxygen is removed from the blood in the capillaries as the blood circulates in the body.

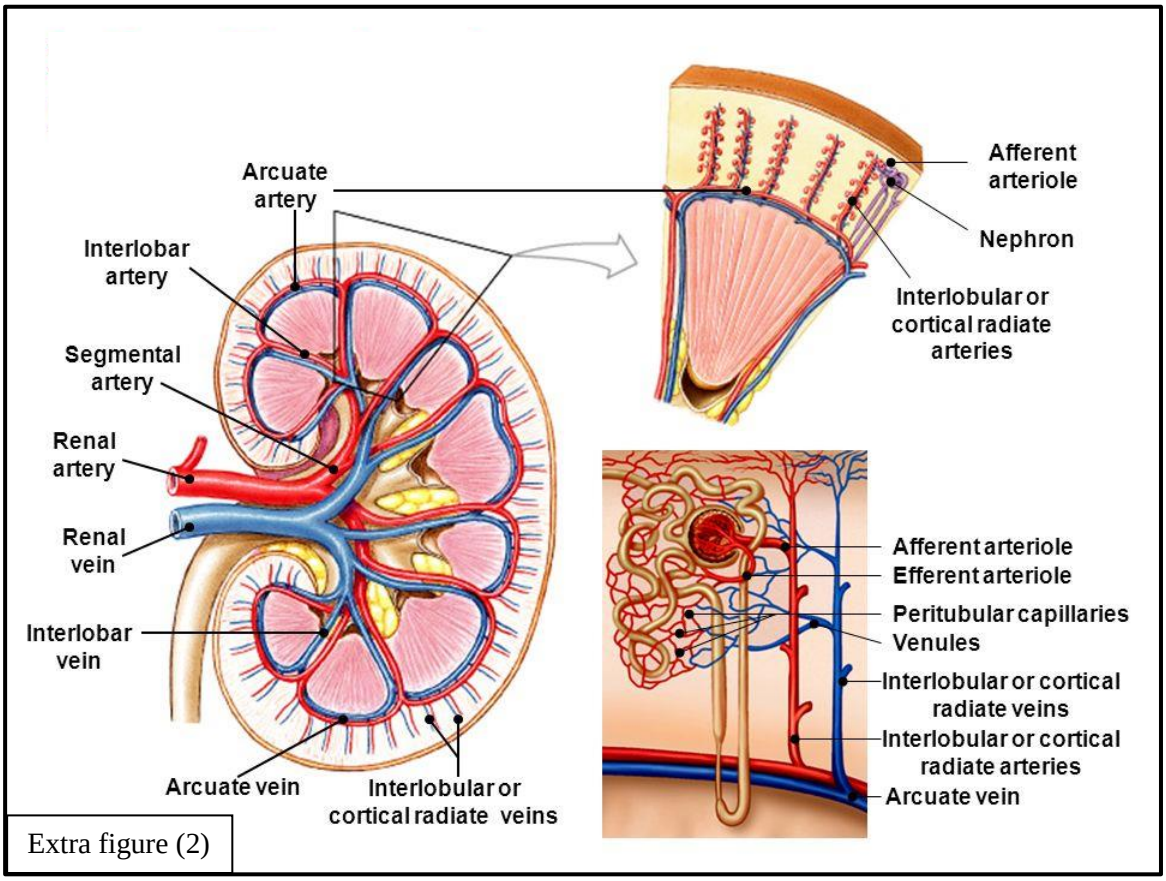
- If the value of the a-vO_2 difference for an organ is small, then this organ is a reconditioning organ (because too much blood goes to the organ, but not only for the purpose of oxygen delivery).
  Example: the kidneys.
- If the a-vO_2 difference is large, then the organ is an essential organ (it receives blood exactly as much as it needs O_2. If it needs more O_2, then more blood should be delivered to it).
  Examples: skeletal muscles, the myocardium (which gets its blood supply through the coronaries).

Additional pieces of information:

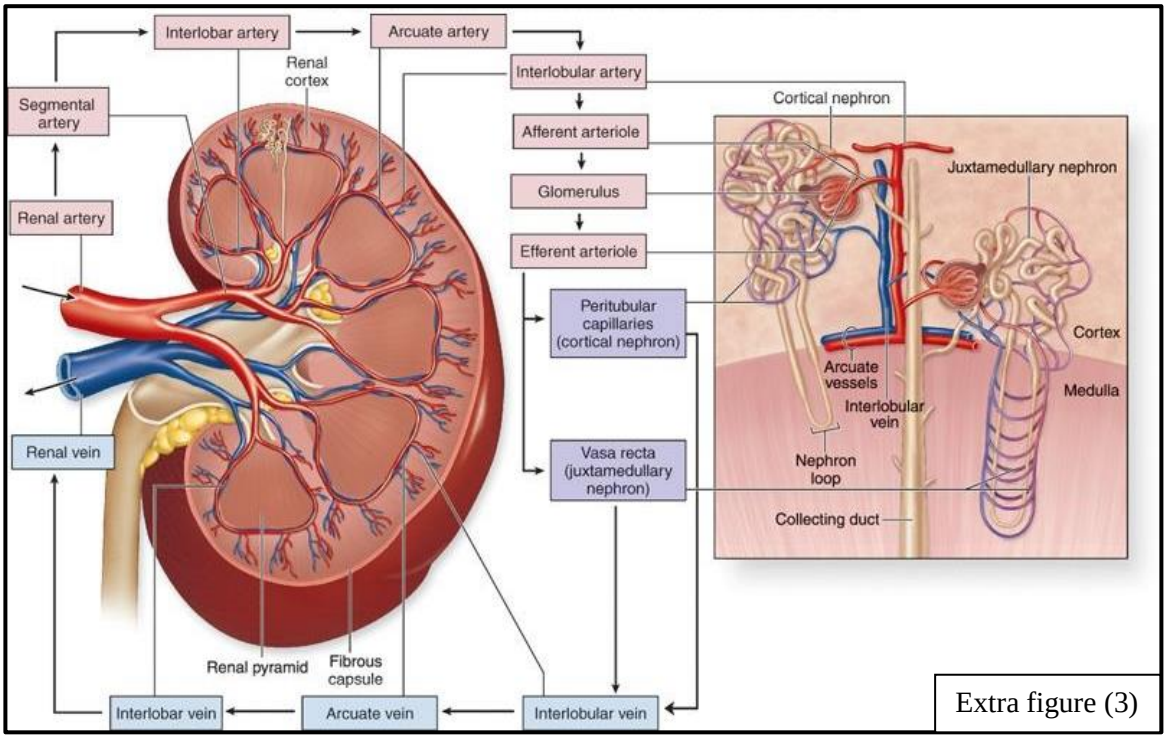
- The reconditioning organs of the body are: intestines, kidneys and skin.
- They are called "reconditioning organs" because they 'refresh' or 'recondition' blood that flows through them.
- These organs usually receive blood flow in excess of their needs.
- **Intestines** recondition blood by picking up digested nutrient molecules from the GI lumen (this action reconditions blood by adding various nutrient levels). The **kidneys** recondition blood by eliminating metabolic wastes and by adjusting water and electrolyte balance. The **skin** eliminates heat.

- How does the blood reach the kidney? *{extra figures (2) and (3)}*

  - Two major renal arteries emerge as branches from the abdominal aorta (one renal artery for each kidney).
  - Once the renal artery enters its corresponding kidney, it divides into segmental arteries.
  - The segmental artery gives interlobar arteries.
  - Then:
    - ➔ arcuate artery
    - ➔ interlobular
    - ➔ afferent arteriole
    - ➔ glomerular capillaries
    - ➔ efferent arteriole
    - ➔ peritubular capillaries
    - ➔ to the veins (back to the venous circulation).



Extra figure (2)



Extra figure (3)

❖ Parts of the nephron

- The kidneys are composed of two million nephrons (one million in each kidney).
- As you already know from the histology lectures with Dr. Faraj, the nephron has many parts. But in this lecture, we will consider the nephron as if it is composed of two parts only. *{figure (1)}*

(A). The first part of the nephron is the "**Ultrafiltration Device**".

- This part is represented by a tuft of capillaries surrounded by a part of the nephron termed as "Bowman's Capsule".
- The process that occurs in this part is "filtration". Fluids from the capillary leave the capillary and enter a space called "Bowman's space"
- Note that both, the afferent and efferent, are ARTERIOLES (the efferent is **not** a venule).
- Filtration is a **passive process**.

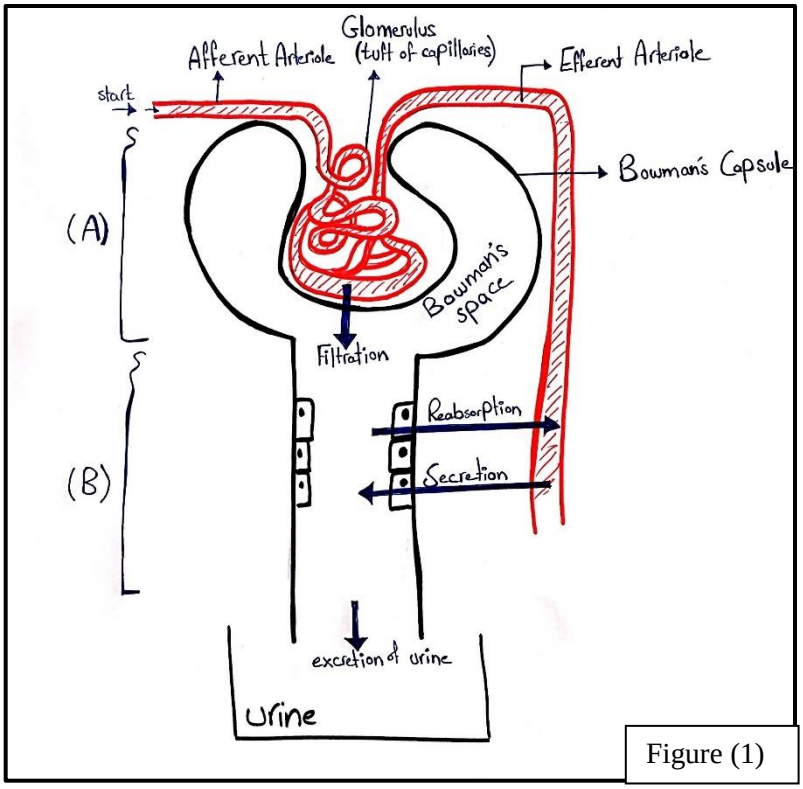


Figure (1)

(B). The second part is "**Modification Device**".

The epithelial cells of this part of the nephron will modify the ultrafiltrate.
Ultrafiltrate modification involves two processes; **Reabsorption** (taking certain substances out of the ultrafiltrate), and **Secretion** (adding certain substances to the ultrafiltrate). The end result is excretion of urine.

Note (mentioned by the Doctor):

The filtration process is considered "Bulk flow", and not diffusion.

Explanation *(additional):*

Diffusion means the net movement down a concentration gradient due to the random motion of individual molecules (note: solutes may move independently of water). On the other hand, **bulk flow** means movement of water and solutes together, due to pressure gradient.

Kidney disease either affects

- Part (A) {the glomerulus and the Bowman's capsule related to it}
  ➔ No proper filtration
  ➔ Decreased GFR
  And in this case, according to the decrease in GFR, we can stage the disease (stage I, II, III, IV, or V)
  In other words, GFR is a tool to classify renal diseases.
  Details in the next lectures
- Part (B) {in the tubule}

For example, nephritis (التهاب الكِلية) could be either "Glomerulonephritis" (affecting the glomeruli) or "Tubulonephritis" (affecting the tubular part)

Another way to describe the renal diseases according to the site is:

- Cortical – if the disease affects glomeruli.
- Medullary – if it affects the tubular part.

Note: the cortex is 1 cm wide and is full of glomeruli, that's why it looks granular. On the other hand, the medulla looks striated because it is full of tubules like loops of Henle, collecting tubules and collecting ducts. The border between the cortex and medualla is called " cortico-medullary border).

Formation of Bowman's space/capsule

- The nephron is about 6 cm long (from its beginning to its end).
- In the beginning, it is a tube, but later in the embryonic life, the tip of the nephron gets invaginated by a tuft of capillaries (the developing glomerulus).
  ➔ Formation of Bowman's capsule and space.
  (we can consider the Bowman's capsule to be the beginning of the nephron. However, don't forget that the structure (histology) of Bowman's capsule is, somehow, different from other parts of the nephron).

Note: anything that exits the capillaries must enter the nephron.

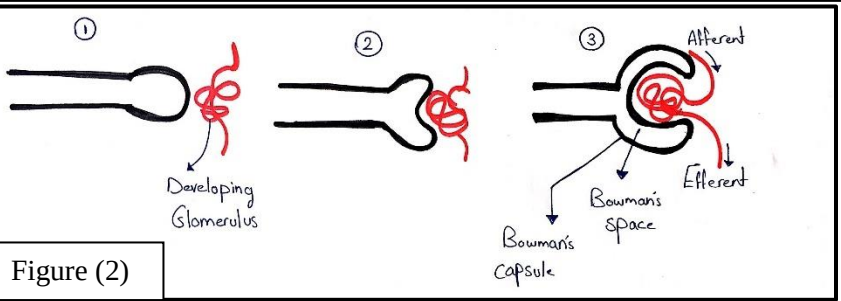


Figure (2)

- The capillaries that emerge from the efferent arteriole are called "peritubular capillaries". And there's interstitium between these capillaries and the tubular part.

- There are two types of nephrons *(go back to extra figure (3)):*
  1. Juxtaglomerular nephrons (15% of the nephrons are of this type).
     Characterized by a long loop of Henle that extends into the medulla.
     ***Note**: in the upcoming lectures, we will study how this long loop of Henle has a significant role in concentration of urine.*
  2. Cortical nephrons (85% of the nephrons are of this type)
     Characterized by a glomerulus residing in the outer most layer of the cortex and a short loop of Henle.

❖ Processes involved in urine formation

Figure (3)

1. **Glomerular Filtration (GF)**
   From the blood of the glomerular capillaries, to the Bowman's space.
   This process is passive.
2. **Tubular Reabsorption (TR)**
   From the tubule, to the blood of the peritubular capillaries.
   This process could be passive or active.
3. **Tubular Secretion (TS)**
   From the peritubular capillaries to the tubule.
   This process is active.
4. **Excretion (of urine)**
   This is the net outcome of the previous three processes.

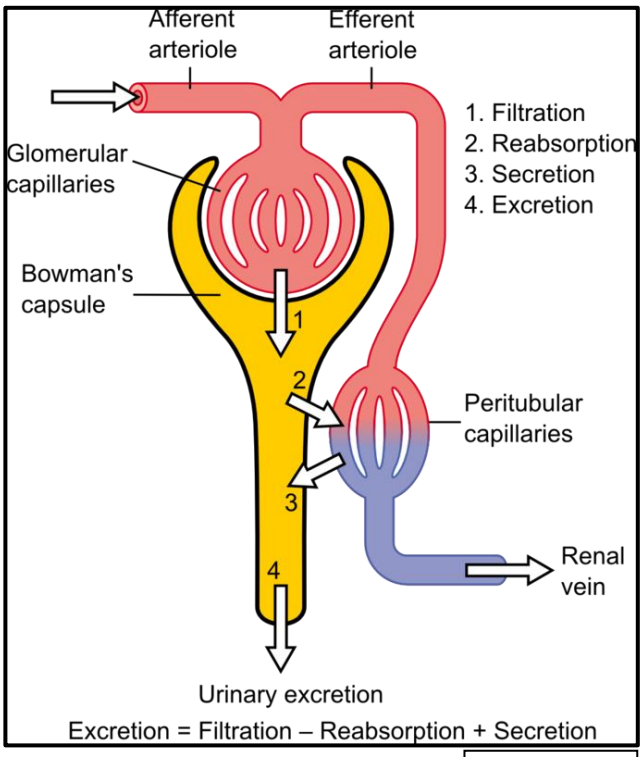


Figure (3)

** Urine Excretion = (Filtration +Secretion) – Reabsorption **

Note: if we take any of the previous processes "per minute", it becomes a rate. For example, Glomerular Filtration **RATE** (GFR) = 125 ml/**min**. (in females, the GFR is 15% less).

For the remaining part of this sheet, and for the next sheet, we are specifically concerned with "Filtration" and certain related measuremnets. This is why we should mention first the main points concerning physiology of filtration.

Note 1:In the following discussion, the numbers are not necessarily 100% accurate. They were given as they are for the purpose of simplicity.

Note 2: the following discussion applies to the 2 million nephrons together, but for simplicity, we study (draw) one nephron.

Figure (4) – glomerular Filtraion

(A). The renal blood flow (RBF) =1250 ml/min (delivered through the afferent arterioles)

- Renal Blood Flow (**RBF**): the volume of blood that goes to both kidneys per minute.
- Renal Plasma Flow (**RPF**): the volume of plasma that goes to both kidneys per minute.

➔ If you know one of them (RBF or RPF) in addition to the hematocrit (Hct), you can calculate the other using the following equation:

$$\mathbf{RBF = \frac{RPF}{(1 - Hct)}}$$

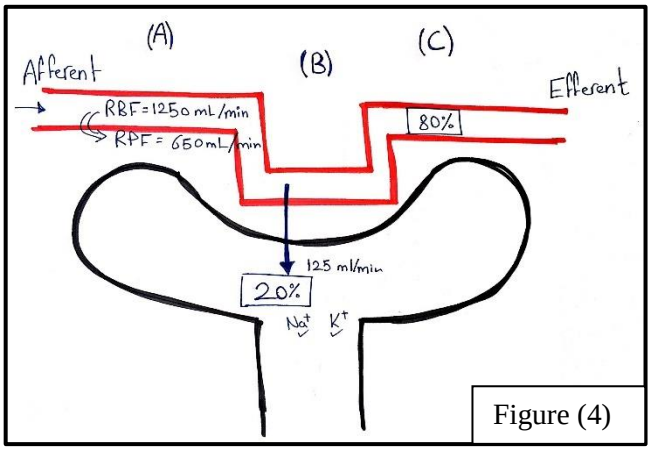


Figure (4)

Let's say that Hematocrit is 45% (0.45)

➔ In this case, 55% will be plasma and thus:
Renal plasma flow (RPF) = 650 ml/min *(it's not exactly 650, but the Doctor used these numbers for simplicity)*

(B). 125 out of the 650 ml will be filtered (1/5 of the RPF will be filtered per minute → 20% of the RPF is filtered)

This 20% represents what we call "Filtration Fraction".

Filtration Fraction = (GFR/RPF) * 100%

The composition of the ultrafiltrate is <u>the same as plasma, except for proteins (they can't pass due to their large size).</u>

For example, the concentrations of Na^+ and K^+ in the filtrate are the same as their concentrations in plasma (145 mmol/L and 4 mmol/L, respectively).

(C). The remaining 80% will continue to the efferent arteriole.

The composition of this part is similar to plasma but with a slightly higher concentration of proteins compared to normal plasma. (because 20% was filtered as plasma except proteins, so the fluid got less without a decrease in proteins → protein concentration gets a little bit higher).

Before continuing to the next section, an important principle must be mentioned.

The final fate of a substance that reaches the kidney through the renal artery is either:

- To exit with urine (excretion)
- Or to reach the renal vein (no excretion)
- Or part of this substance gets excreted, and the other part reaches the renal vein.

Amount (entered) = Amount (excreted) + Amount (that left the kidney back to the renal vein)

Important note: for the previous to be true, the substance we're talking about must meet the following conditions:

- NOT made by the kidneys (the substance will be infused from an **exogenous** source).
- Does NOT get accumulated in the kidney, and
- NOT metabolized (degraded) by the kidney.

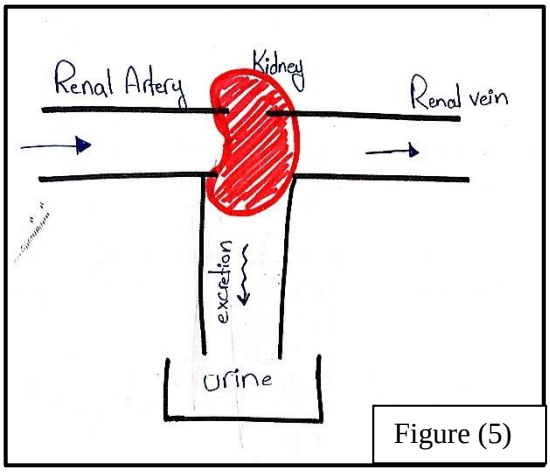


Figure (5)

Examples:

- If the amount entered equals 10 mg/min, and the amount excreted is 5 mg/min, then the amount that reaches the renal vein of this substance is 5mg/min.
- If a substance gets completely excreted → its amount in the renal vein will be zero.
- If nothing of the substance gets excreted → its amount in the renal vein will be the same as the original amount that entered the kidney.

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*Page (9) discussed the **normal physiology of filtration**. However, in the following discussion please keep in your mind that we will be talking about measurements and tests that we can carry out (how can **we measure** RPF and RBF?)*

❖ How can we measure RPF and RBF?

***We will learn a specific technique to measure RPF, and from the equation in page (9), we can then calculate RBF.*

To measure RPF, we use a specific substance (let's call it substance X)
Substance X should meet many conditions. The first three are extremely important:

1. Substance X should be **freely filtered**.
   This means that its concentration in the filtrate will be the same as its concentration in the plasma.
   Filtration fraction is 20%.
2. **Not reabsorbed.**
   This means that the fate of any part of substance X that gets filtered will be excreted through urine.
3. The 80% that remains not filtered should be **completely secreted** once it comes around the nephron.
   ➔ The outcome of the previous conditions is:
   100% of substance X is excreted (20% due to filtration, and 80% due to secretion).

Substance X should be completely cleaned from the blood once it enters the kidney.

In other words, **the concentration of substance X in the renal vein is ZERO**, and the "extraction ratio" by the kidneys is 100%.

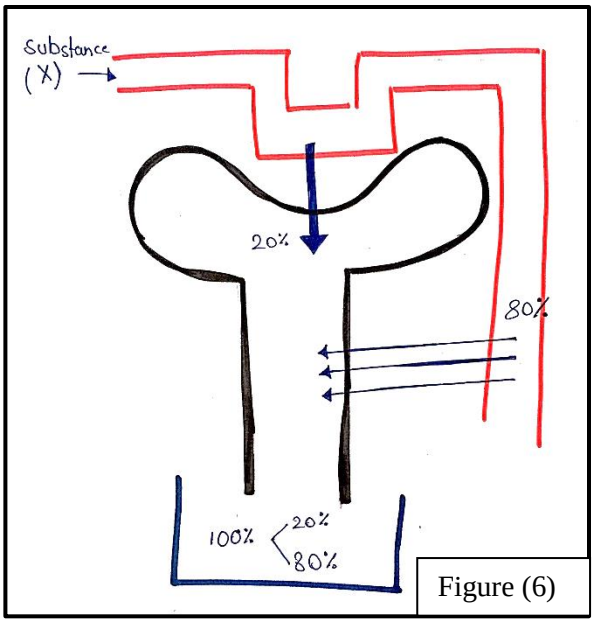


Figure (6)

4. Substance X is NOT made by the kidney (it will be infused from an exogenous source), does NOT get accumulated in the kidney, and NOT metabolized (degraded) by the kidney.

Steps (method):

- Since the amount of substance X in the renal vein is zero, the amount (number of milligrams) that enter the kidney per minute will equal the amount (number of milligrams) leaving through the urine per minute. *(according to the "conservation of mass" law)*
  Or we can say:

  The amount excreted/min = the amount provided for excretion/min

  - The amount provided for excretion:
    {each minute, how many milligrams enter the kidney?} → RPF * P_x
  - The amount excreted:
    {each minute, how many milligrams leave the kidney through urine?}→ $\dot{V} * U_x$

The amount of a substance in a solution depends on:

1. The volume
2. The concentration of the substance

Amount = Volume * Concentration

For example, if the concentration of a substance in the plasma is 1mg/ml, and the volume of plasma entering the kidney per minute (RPF) is 650:

→The amount that enters the kidney of this substance every minute is:

650 *1 = 650 mg.

P_x: concentration of substance X in plasma

U_x: concentration of substance X in urine

RPF: renal plasma flow (ml/min)

$\dot{V}$: urine output (ml/min)

Or any (volume/time) unit, but should be the same as the one you use for RPF.

**pay attention to the units in the exam.*

- RPF * Px = $\dot{V}$ * Ux

  → RPF = (U_x/P_x) * $\dot{V}$

  - RPF → what we want to find.
  - Px → we can know it by taking a sample of blood from any peripheral vein.
  - $\dot{V}$ → we can know it by 24-hour urine collection.
  - Ux → we can know it by using the urine sample we took from the patient.

- To find out the value of the urine output ($\dot{V}$), we should use a 24-h urine collection and then calculate the urine output per minute.

  For example, if the amount collected in 24 hours equals 2000 ml:

  → Per hour: 2000/24
  → Per minute: 2000 / (24*60) = 1.4 ml

- Why do we use a 24-h collection and then take the average?

  Because the amount of urine that the individual produces differs from one time to the other, so taking the urine produced in a single period of time ("spot urine") will not be accurate. On the other hand, when we calculate the average of a 24-h collection, the margin of error will be minimal.

- After you calculate the RPF, you can use the following equation to calculate the RBF:

$$RBF = \frac{RPF}{(1 - Hct)}$$

*You can get the Hct value (PCV), by taking a blood sample from the patient and then centrifuging it.

Clearance *(a very important term that you must understand)*

- To understand what we mean by "clearance", let's assume that you were given 650 ml containing substance (A)
  - If you returned back the 650 ml totally free of substance (A), this menas you "cleaned" the 650 ml (you cleared the 650 ml from substance (A)).
  - If you returned the 650 ml containing the same original amount of substance (A), this means you cleaned zero mL (clearance is zero mL).
  - If you returned back the 650 ml, with half of the amount of substance (A) removed, then you cleaned 325 ml (clearance is 325 ml).
- Clearance is the **volume** of plasma cleaned from a certain substance per minute.
  And to be more accurate, we can say "Clearance is the **volume** -of plasma- that provides a certain substance for excretion, per minute".

  ➔ The unit of clearance : **volume per unit time** (for example: ml/min)

- Now, let's go back to our special substance (X):
  We said that the amount of X that enters the kidney, will be completely excreted in urine.
  If the RPF = 650 ml/min:
  →The volume of plasma that provides X for excretion per minute is 650 ml.
  →Clearance of substance X = 650 ml/min.

Note: measuring the RPF (and then the RBF) using the previous method is NOT a routine clinical test, because it's very hard and complex.

The difficulty and complexity stem from many factors, the most important of which are:

- There's no substance in the human body that gets completely cleaned once it enters the kidney. This means we have to inject an exogenous substance.
- We need to stabilize the plasma level of the substance injected, and this takes a considerable period of time.

What do we mean by "stabilize"?
Amount excreted in urine = amount infused into the blood
→This means that we need a pump, to maintain the concentration of substance X in the plasma stable.

- During the period in which the plasma level of the substance is stable, we need to collect urine.
  **A patient in a clinic will not accept such time-consuming procedures.

This test is performed only for research and academic purposes, not for clinical purposes. (you will not see it during your clinical practice. However, you will be asked about it in the exam).

After we have explained the principles and the steps, we shall reveal what substance X is …

The substance used is Para-amino-hippuric acid (PAH)

PAH gets completely cleaned, but there's a problem.

Before explaining what this problem is, Dr. Yanal reviewed some basic principles.

- Filtration is passive.
- Reabsorption is both (could be passive or active).
- Secretion is active.

- To describe a process as being "**active**", certain criteria must be met (5 criteria to be accurate) but here, we are concerned with only one of them, which is "The Saturation Phenomenon".
- Comparison between passive and active transport:
  - If we plot the concentration of substance X on the x-axis, and the diffusion of X (Jx) on the y-axis, a passive diffusion curve will be represented by a line, similar to *figure (7).*
    - ➔ The more the concentration, the more the diffusion.
  - On the other hand, if the process is active, the curve will be similar to *figure (8).*

    Note the presence of T_{max} (maximum transport, cannot be exceeded). This is due to the phenomenon of saturation.

    Extra: at a certain concentration, all solute-binding sites of transporters will be occupied, thus, additional amounts of the solute will not be transported (the flux (J) curve, reaches a plateau at T_{max}).

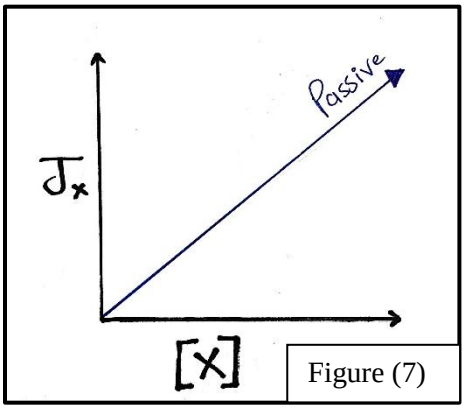


Figure (7)

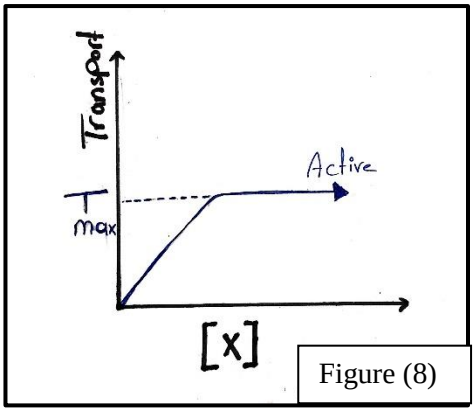


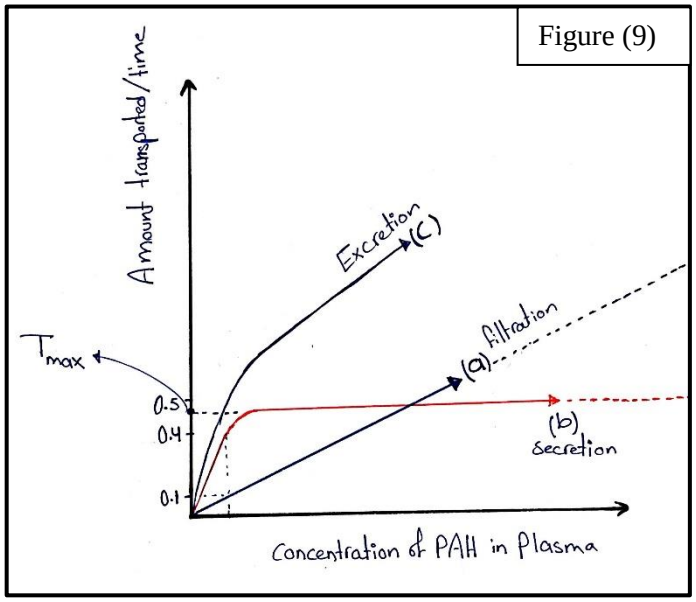
Figure (8)

Now, what is the problem with PAH (substance X)?

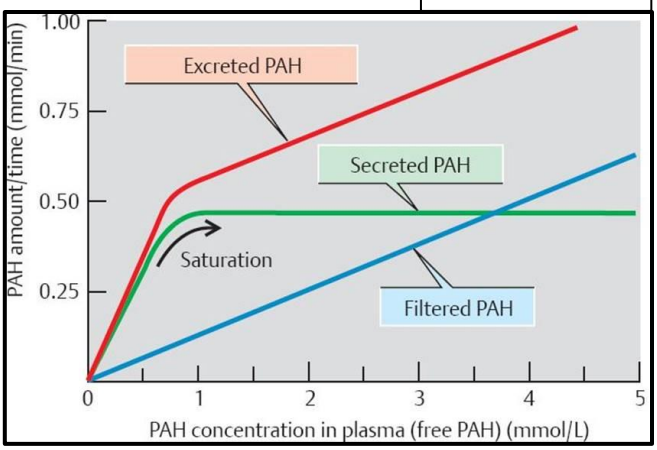
- We said that the excretion of substance X is the result of filtration (passive) and secretion (active).

- Look at ***figure (9)***
  - **<u>Curve (a)</u>:** represents the filtered load (or let's say "filtration").
    Filtered load = amount of X (PAH) filtered per minute.
    The more the concentration, the more the amount filtered per minute (because filtration is passive). *Note that it's <u>linear</u>.*
  - **<u>Curve (b)</u>**: represents the secretion curve.
    Note that:
    1. The secretion curve is to the left (compared to the filtration curve). Why?
       Because we said that the amount secreted of X (80%) is 4 times the amount filtered (20%). For example, if a certain concentration causes filtration of 0.1 mmol/min, the same concentration will cause secretion of 0.4 mmol/min, and the sum will be 0.5 (excretion).
       Note: the numbers are not accurate.
    2. At a certain concentration, the secretion curve reaches a plateau. Beyond this point, an increase in the concentration will not lead to an increase in the secretion
    3. If we extend the line of filtration, and the curve of secretion → after a while, the secretion will get so small (negligible) compared to the filtration.
  - **<u>Curve (c)</u>**: represents the excretion curve (the result of the summation of the two curves).

Figure (9)



Extra Figure (4)

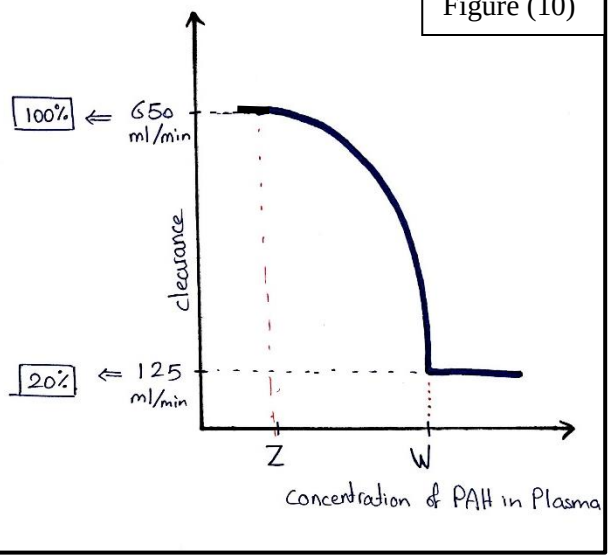


- We conclude from the previous curves that the problem with PAH is that if you infuse too much PAH (beyond the capacity of the tubules), not all of the PAH will be secreted (because you reached T_{max}) and some of it will go back to the renal vein. In this way, you will be breaking the conditions.
  Remember: to be able to -correctly- use the steps and equations we mentioned, all the necessary conditions must be met.
  On the other hand, if you use a small concentration of PAH (before reaching T_{max}), you will not be breaking the conditions.

- For example: let's assume that injection of 0.1 mg/ml of substance X guarantees that the entire plasma (650 ml) will be cleared, while any concentration higher than 0.1 is a concentration beyond the point that corresponds to T_{max}:
  - When the concentration is 0.1 or less → clearance will be 100%
  - As the concentration increases beyond 0.1, the clearance decreases (90%, 80%....., 30%, 20%). Why do you think we stopped at 20%?
    Because this 20% cleared plasma results from FILTRATION, and filtration is passive. → <u>the 20% clearance is guaranteed always</u>, even if the concentration increases dramatically to the extent that it causes secretion to be too small or negligible compared to filtration *(go back to figure (9) - curve (b) - point 3.)*

- To make the previous easier to imagine, let's plot the concentration of PAH in plasma on the x-axis, and the clearance of PAH on the y-axis. The result will be *figure (10):*
  - The maximum clearance according to our example is 650 ml/min. (100% clearance → clearance of all the plasma from PAH).
    Let's assume that this 100% clearance is achieved at a certain concentration of PAH that is equal to Z.
  - If you increase the concentration of PAH beyond point Z, clearance will decrease until you reach a point after which increasing the concentration will not result in a decrease in clearance (point W in the figure).

Figure (10)



  - Note that at concentration equal to W or more, the clearance = 125 ml/min (which corresponds to the 20% that is due to filtration).

➔ The more the concentration of PAH in plasma, the less the clearance, until you reach a plateau that is equal to the GFR.

- From the previous discussion, we conclude that an additional condition must be met for the use of substance X:
  You have to know what's the T_{max} of the substance, and make sure that the amount that reaches the peritubular capillaries does not result in reaching T_{max} (to guarantee complete secretion, end thus, 100% clearance {100% extraction}).

At the end of the lecture, Dr. Yanal summarized the measurements section by the following key points:

- You should be able to know how to measure RPF and RBF, but don't forget that these two tests are NOT routine clinical tests.
- If you measure RPF, you can calculate RBF.
- How to measure RPF?
  - We use a substance which is completely removed (cleaned/extracted) once it enters the kidney.
  - Using the law of "conservation of mass" we concluded that:
    The amount excreted/min = the amount provided for excretion/min

$$\dot{V} * Ux = RPF * Px$$

- The substance we can use is PAH. However, certain conditions must be met. The most important of these is:
  The delivered concentration should be below the concentration that results in reaching T_{max}. Otherwise, the result we get will <u>underestimate RPF</u>
  {Not all plasma will be cleared, so the clearance in this case will underestimate RPF (because part of the substance will reach the renal vein, thus, the number we get by calculations will be less than the true RPF)}.

I apologize for any mistake I may have made.

Wish you all best of luck :D